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Characterization of the potato mitochondrial transcription unit containing 'native' *trnS* (GCU), *trnF* (GAA) and *trnP* (UGG)

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Abstract

In order to identify the sequences promoting the expression of plant mitochondrial tRNA genes, we have characterized the *trnS* (GCU), *trnF* (GAA) and *trnP* (UGG) transcription unit of the potato mitochondrial genome. These three tRNA genes were shown to be co-transcribed as a 1800 nt long primary transcript. The transcription initiation site located 305 to 312 nt upstream of *trnS* is surrounded by a purine-rich region but does not contain the consensus motif proposed as a promoter element in dicotyledonous plants. Differential labelling of potato mitochondrial RNA with either guanylyltransferase or T4 polynucleotide kinase suggests that this site corresponds to the unique functional region responsible for the transcription of these three tRNA genes. The initiation site recently found upstream of *Oenothera* mitochondrial *trnF* does not seem to be used in potato mitochondria, although a very similar sequence is present 317 nt upstream of the corresponding potato gene. Major processing sites were identified at the 3′ end of each tRNA gene. Another processing site, surrounded by a double hairpin structure, is located 498 nt downstream of *trnP* in stretch of 10 A residues. As judged from northern experiments, this region is close to the determination site of this transcription unit.

Introduction

The expression of mitochondrial tRNA genes in plants is of special interest because they have two different genetic origins. Some are called 'native' and are thought to be derived from the bacterium which was the progenitor of mitochondria, some others are called 'chloroplast-like' because they are present in promiscuous chloroplastic DNA sequences which were inserted into the mitochondrial genome during evolution [33]. Although most of the tRNA genes having a chloroplastic origin are silent, some of them are expressed in plant mitochondria (for a review, see [20]) but

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers X87675 and X87676.

very few informations are available about their transcription. In particular, it is interesting to note that there is no real rule to deduce from the sequence level whether a 'chloroplast-like' tRNA gene will be expressed or not. For instance, although 'chloroplast-like' *trnP* and *trnW* are only separated by about 150 bp in several plant mitochondrial genomes [14, 18], the first one is not expressed whereas the second one is expressed. In the case of the 'chloroplast-like' *trnH*, this gene is surrounded by typical chloroplastic sequences in the maize mitochondrial genome [12] whereas in sunflower [1], *Oenothera* [2] and potato (Maréchal-Drouard *et al.*, unpublished results) the flanking sequences of this gene are very different from their chloroplastic counterparts, but transcription of this 'chloroplast-like' *trnH* occurs in all these plant species. Up to now, it is not known whether the transcription of these 'chloroplast-like' tRNA genes is due to different types of tRNA gene promoter elements or to their insertion in already transcribed regions of the plant mitochondrial genomes.

In order to get more insight on the expression of these 'chloroplast-like' tRNA genes, it is also necessary to learn more about the transcription of the 'native' plant mitochondrial tRNA genes. Joyce *et al.* [13] first identified a purine-rich motif in a conserved position upstream of wheat mitochondrial tRNA genes and proposed that this sequence could play a role in their expression. This sequence was subsequently found upstream of some mitochondrial tRNA genes in other plant species (for a review, see [20]), but until now, there is no direct evidence that such a motif could play a role in the expression of mitochondrial tRNA genes. The first region shown to be involved in the transcription of a plant mitochondrial tRNA gene, the *Oenothera* mitochondrial *trnF*(GAA), was described only recently by Binder and Brennicke [3]. This transcription initiation site localized in a purine-rich region was shown to contain the consensus motif CRTAa-GaGA found in putative mitochondrial mRNA and rRNA promoters of dicotyledonous plants [4].

In this report, we present results concerning the transcription unit containing 'native' *trnS*(GCU), *trnF*(GAA) and *trnP*(UGG) present as single copy in the mitochondrial genome of potato [19]. These three genes were shown to be co-transcribed giving rise to a primary transcript of about 1800 nt long. Although the transcription origin, mapped 305 to 312 nt upstream of *trnS*, corresponds to a purine-rich sequence, this region does not fit with the consensus motif CRTAa-GaGA proposed as a promoter region. A comparison between this potato transcription unit and the *Oenothera* transcription unit containing *trnF*-(GAA) will be discussed. Several smaller transcripts probably corresponding to processing intermediates were identified and the termination site was tentatively located about 800 nt downstream of *trnP*.

Material and methods

Extraction of potato mitochondrial nucleic acids

Isolation of potato (*Solanum tuberosum*) mitochondrial DNA was performed under conditions previously described [19]. For RNA extraction, mitochondria purified from potato tubers according to [26] were lysed in the presence of 10 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 1% (w/v) SDS. Lysis was immediately followed by an extraction with one volume of water-saturated phenol. The aqueous phase was recovered by a 15 min centrifugation step in a microcentrifuge at 13000 rpm. Nucleic acids were precipitated in the presence of 0.1 vol of 1 M sodium acetate pH 4.8 and 2.5 vol of ethanol for at least 1 h at −20 °C. After centrifugation as above, nucleic acids were dissolved in water and LiCl was added to a final concentration of 2 M. The sample was kept on ice for at least 30 min and after centrifugation DNA, 5S rRNA and tRNAs were recovered in the supernatant whereas mRNAs, precursor RNAs and large rRNAs were recovered from the pellet and will be described as LiCl-precipitating RNA. To eliminate LiCl, supernatant and pellet redissolved in water were submitted separately to two classical ethanol precipitation.

Gene-specific clones, cloning and sequencing

The cloning of a 2.8 bp potato mitochondrial DNA fragment containing the *trnS-trnF-trnP* cluster has been previously described [19]. The upstream sequence as well as internal DNA fragments containing part of this transcription unit were cloned and subcloned in pBluescript KS+ or pUC18 vectors following standard protocols [30]. DNA sequencing was performed by the dideoxyribonucleotide chain termination method.

In vitro *capping*

In vitro capping reactions were carried out essentially as described by Binder and Brennicke [3] in a reaction volume of 40 μl containing 50 μg of potato mitochondrial LiCl-precipitating RNA, 50 mM Tris-HCl pH 7.9, 80 units of RNase inhibitor, 1 μM GTP, 250 μCi [α-^{32}P]GTP (3000 Ci/mmol) and 30 units of guanylyltransferase (Gibco-BRL). After 1 h incubation at 37 °C, the reaction was stopped by digestion with 20 μg of proteinase K and 0.5% (w/v) SDS for 15 min at 37 °C. Capped RNA was purified by one phenol/chloroform extraction and unincorporated nucleotides were removed by passing through a 1 ml Sephadex G-50 spun column as described [21].

RNase protection, primer extension and RT-PCR

RNase protection was based on Goodall *et al.* [8] except for modifications in the denaturation step: the riboprobe (radioactively labelled or not) and total mitochondrial RNA (radioactively capped or not) were denatured for 10 min at 75 °C and hybridization was allowed to proceed for 3 to 4 h at 45 °C.

For primer extension, 2 to 5 μg of LiCl-precipitating RNA were added to 5 ng of 5′-end-labelled oligonucleotide. After denaturation for 10 min at 75 °C, hybridization was allowed to proceed for 3 to 4 h at 47 °C in the buffer supplied by the manufacturer of the enzyme (Promega) and the extension reaction was also performed according to this protocol. After ethanol precipitation, reaction products were analysed on 6% polyacrylamide sequencing gels.

For RT-PCR experiments, reverse transcription was performed as described above for primer extension experiments except that LiCl-precipitating RNA was first treated three times with RNase-free DNase (Pharmacia) under conditions supplied by the manufacturer. One fifth of the reaction was taken for PCR amplification using buffer supplied with *Taq* Polymerase (Appligene) in the presence of 0.5 μg of each oligonucleotide and 200 μM of each dNTP. The denaturation, annealing and extension times were 80 s for each step at 94 °C, 50 °C and 72 °C respectively. Amplifications were carried out with 25 or 50 cycles on a Crocodile II Appligene apparatus.

Kinase reaction

Up to 50 μg of LiCl-precipitating mitochondrial RNA was labelled in a 40 μl reaction medium containing the buffer supplied with the enzyme (Eurogentec), 40 U of polynucleotidekinase, 80 U of RNase inhibitor and 100 μCi[γ-^{32}P]ATP (3000 Ci/mmol). The reaction was incubated for 1 h at 37 °C and then treated as described above for the *in vitro* capping of RNA.

Misceallenous methods

To generate the riboprobes used for the RNase protection experiments, recombinant pBluescript vectors were linearized with the appropriate restriction enzymes and transcribed *in vitro* using T7 or T3 RNA polymerases in the presence or not of 10 μCi [α-^{32}P]UTP (3000 Ci/mmol) according to manufacturer's instructions (Gibco BRL).

DNA fractionated on agarose gel and RNA fractionated on agarose-formaldehyde gel were transferred on Hybond N+ and Hybond N nylon membranes respectively according to Amersham's instructions. Hybridizations on Southern blots were performed using standard protocols

[30] when oligonucleotides were used as probes and according to Binder and Brennicke [3] when capped or phosphorylated RNA was used as probes, except that the hybridization and prehybridization buffers were prepared without dextranesulphate but with 100 μg/ml of salmon sperm DNA. For northern blots, hybridizations and prehybridizations were done in 50% formamide, 5× SSC, 1% SDS and 100 μg/ml of salmon sperm DNA at 42 °C with PCR-generated DNA probes. Filters were washed for 10 min in 1× SSC, 0.1% SDS and for 15 min in 0.2× SSC, 0.1% SDS at 60 °C.

For PCR-generated DNA probes, reactions were performed as described above for RT-PCR except that 100 ng of recombinant plasmid were used as a template and 10 μCi [α-^{32}P]dCTP (3000 Ci/mmol) were added in the reaction mixture.

Labelling of oligonucleotides at their 5′ end was performed in the presence of 25 μCi [γ-^{32}P]ATP using buffer and conditions supplied by the manufacturer of the polynucleotidekinase (Eurogentec).

The oligonucleotides used in these experiments were the followings:
(1): 5′-GGAGGTATGGCTGAGTGGC-3′
(2): 5′-AGTAGGGATCCGGGCGCTCTTCG-ATGAAGAAAACAG-3′
(3): 5′-GTTCAGGTAGCTCAGCTGGTTAG-AGC-3′
(4): 5′-TCTATATGCTTCGGGGTGGTGA-GA-3′
(5): 5′-TTCTATGAACACCCCACCACTG-AA-3′
(6): 5′-GTCGATTTAGCAAACCAATG-3′
(7): 5′-TCGCCCGCCCGTTTCATTTCCGT-AC-3′
(8): 5′-CAACGGGCTCTCCGCTCCTAG-3′
(9): 5′-AGTACGGATCCTGCTTAGAAGTG-GATTCGAACCACTG-3′
(10): 5′-GCGCTGACCAGACTGCGCTACA-CCTCG-3′
(11): 5′-TCAAGGTGACAGGATTCGAACC-TA-3′
(12): 5′-GATTGCTCTTGTTGATACAGTT-TC-3′
(13): 5′-GGGAAGGGCTGTTATGTAGG-CG-3′
(14): 5′-AAAGCAAACTAAGAGCAGGGA-AGG-3′.

Results

Evidence for the co-transcription of trnS, trnF *and* trnP *in potato mitochondria*

As already shown, these three tRNA genes found as single copy in the potato mitochondrial genome are relatively close to each other ([19] and Fig. 1) and it was therefore tempting to assume that they could be co-transcribed. This hypothesis was strongly supported by northern blot analyses carried out with very sensitive PCR-generated probes specific for *trnS*, *trnF* or *trnP* where, in all cases, the longest transcript detected has the same size of about 1800 nt (Fig. 2). The real proof that these three tRNA genes belong to the same transcription unit was obtained by RT-PCR experiments (Fig. 2). Using two primers corresponding to sequences in the coding regions of *trnS* and *trnP* respectively, a fragment of the expected size (about 800 nt) was amplified. In control experiments, no amplification product could be seen when the RNA sample was subjected to RNase treatment prior to reverse transcription (Fig. 2) or when no reverse transcriptase was added (data not shown).

Mapping and cloning of the transcription initiation site

The 2.8 kb *Eco*RI potato mitochondrial DNA fragment containing *trnS*(GCU), *trnF*(GAA) and *trnP*(UGG) previously described [19] is schematically represented as clone a in Fig. 1. A partial sequence of this clone (from the *Eco*RI site upstream of *trnS* to the *Acc*I site) was already accessible under the EMBL accession number X73286.

In order to look for a putative initiation site involved in the co-transcription of these three

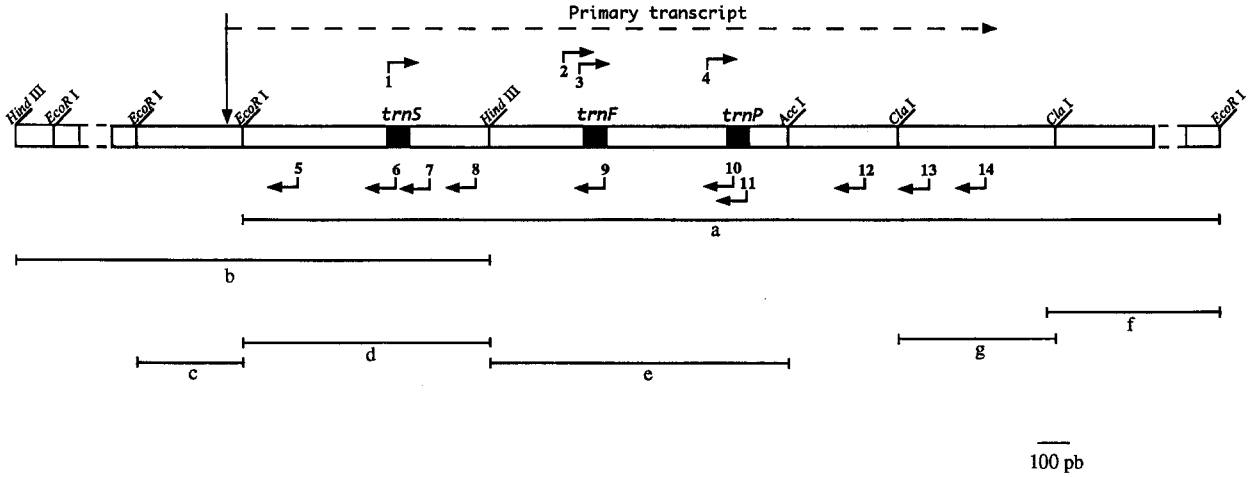

Fig. 1. Partial restriction map of a 4.3 kb DNA fragment containing the potato mitochondrial *trnS*(GCU), *trnF*(GAA) and *trnP*(UGG). Black boxes represent the tRNA genes. Oligonucleotides used for primer extensions, PCR and RT-PCR experiments are numbered from 1 to 14. Orientation of each oligonucleotide is indicated by an arrow. The clones and subclones used in this study are indicated by letters from a to g. The long vertical arrow corresponds to the location of the initiation site.

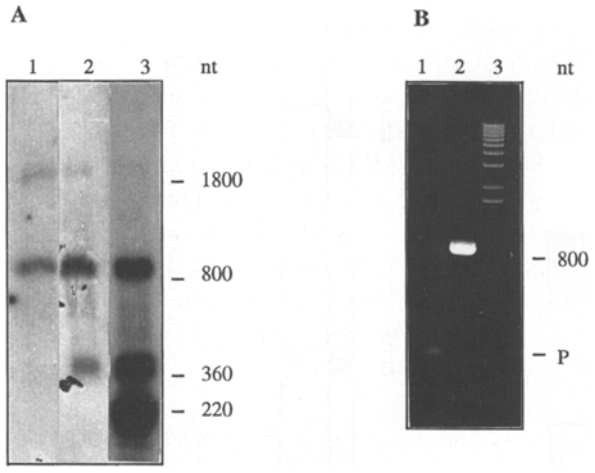

Fig. 2. A. Northern blot analysis of the *trnS*, *trnF* and *trnP* transcription unit. 5 μg of potato mitochondrial RNA are loaded per lane. PCR amplified probes are specific to *trnS* (oligonucleotides 1 and 7 as primers) in lane 1, *trnF* (oligonucleotides 3 and 9) in lane 2 and *trnP* (oligonucleotides 4 and 11) in lane 3. Sizes of the major transcripts are indicated. B. Analysis on agarose gel of the RT-PCR amplification product (lane 2) obtained with oligonucleotides 1 and 10 as primers (see Fig. 1). In lane 1, the RNA was treated with RNase A prior to reverse transcription. Lane 3: 1 kb ladder (Gibco-BRL). Size of the amplification product is indicated. P, migration of the primers. In A and B, the numbering of the oligonucleotides is according to Fig. 1.

tRNA genes, we performed a primer extension experiment with an oligonucleotide (oligonucleotide 6 in Fig. 1) specific for the upstream tRNA gene, namely *trnS*. A strong stop was detected about 300 nt relative to the first nucleotide of *trnS*, corresponding to a region 45 to 50 nt upstream of the *Eco*RI cloning site. Assuming that this strong band could correspond to the initiation site, we cloned a 2.2 kb *Hin*dIII potato mitochondrial DNA fragment (clone b on Fig. 1) overlapping the 2.8 kb *Eco*RI DNA fragment and containing *trnS* and the putative promoter region. A partial restriction map of this DNA fragment was constructed and various subclones prepared (Fig. 1). The 0.45 kb *Eco*RI fragment (subclone c on Fig. 1) adjacent to the 2.8 kb *Eco*RI DNA fragment was sequenced and is accessible under EMBL accession number X87675. Primer extension experiments with oligonucleotide 6 but using clone b as a ladder allowed us to localize the putative initiation site (Fig. 3) in a purine-rich region. The same result was obtained with oligonucleotide 5 (data not shown). As the length of the extension product was varying from one experiment to the other, this region was localized 305 to 312 nt upstream of *trnS*.

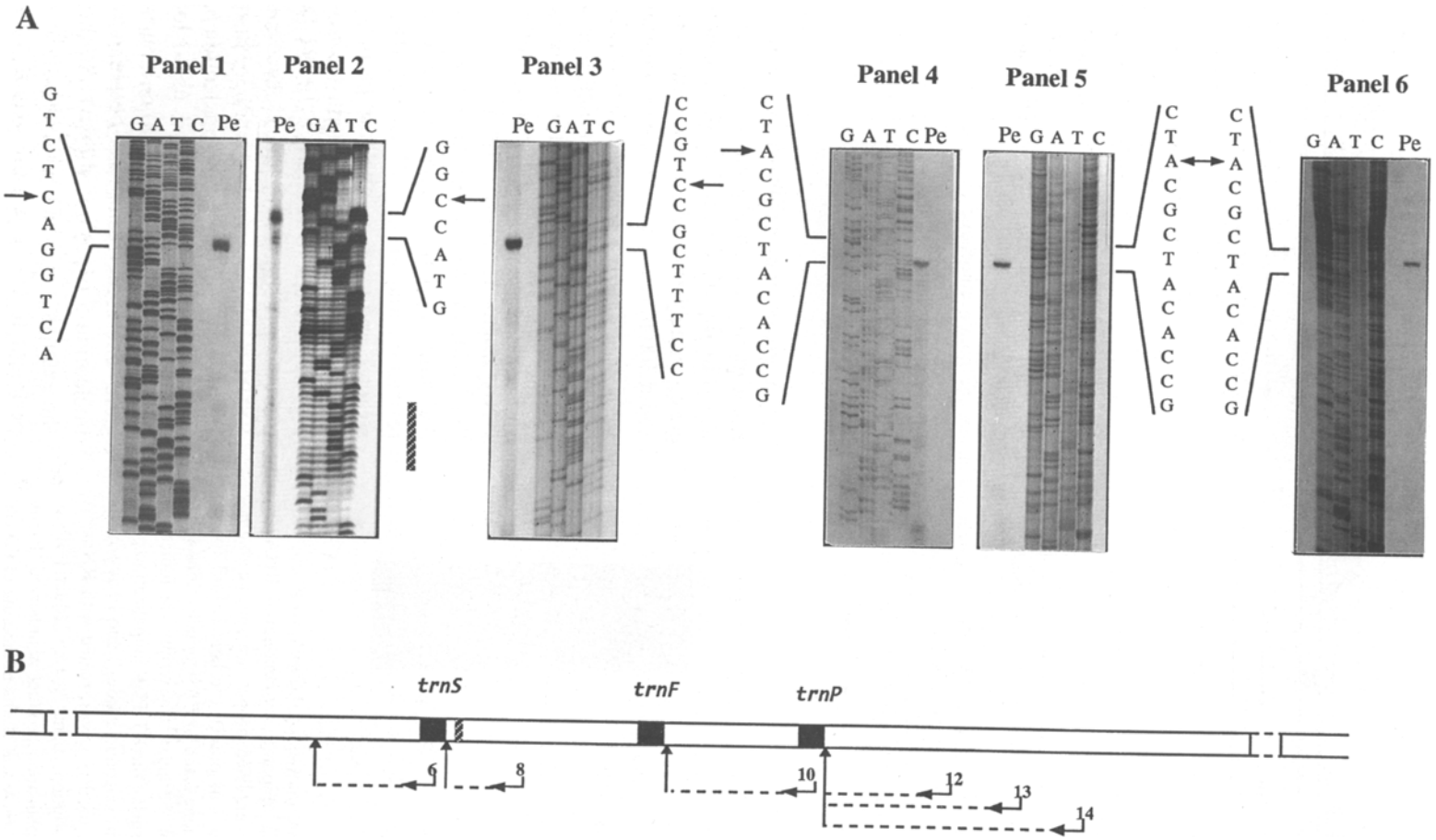

Fig. 3. A. Primer extension experiments using oligonucleotide 6 in panel 1, 8 in panel 2, 10 in panel 3, 12 in panel 4, 13 in panel 5 and 14 in panel 6. The numbering of the oligonucleotides is according to Fig. 1. Sequencing reactions performed with the same oligonucleotides were used as ladder. Arrows indicate the nucleotide position of the 5′ end of the extension product. The dashed box shown in panel 2 corresponds to the sequence which is very similar to the transcription initiation region found upstream of *Oenothera* mitochondrial *trnF* [3]. B. Schematic representation of the results.

To obtain evidence that this site really functions as a transcription origin we carried out *in vitro* capping experiments with potato mitochondrial RNA in combination with ribonuclease protection assays [34]. Organellar primary transcripts have a free triphosphate group at their 5′ end, so that these unprocessed RNAs can be specifically capped *in vitro* with [α-^{32}P]GTP by guanylyltransferase. When capped RNA was hybridized with the antisense riboprobe obtained by *in vitro* transcription of linearized clone c and digested with single-strand-specific ribonucleases, a protected band of 40 nt corresponding to the 5′ end determined by primer extension was detected (Fig. 4). In control experiments, no protected product was obtained when no antisense RNA was added or, when it was replaced by the corresponding sense transcript (data not shown). These data provide the final evidence that this region functions as an initiation site for the co-transcription of the potato mitochondrial *trnS*, *trnF* and *trnP*. As it is now well known that in some plants (e.g. rice) alternative promoters can exist [24], it was of interest to check whether other regions could be responsible for the expression of this transcription unit. For this purpose, other ribonuclease protection assays on capped RNA were performed. When antisense riboprobes generated by *in vitro* transcription of clones d or e (Fig. 1) were used, we did not detect any protected band (data not shown), suggesting that no other transcription site was present up to *trnP*. Furthermore, using a series of primer extension experiments, only processing intermediates were identified and we failed to detect any other putative initiation site in the spacing regions (Fig. 3). In agreement with these results are the data obtained from Southern experiments (Fig. 4). Phosphorylated mitochondrial RNA hybridized with clones d and e which correspond to processed RNA sequences of the 1800 nt primary transcript and did not hybridize with clone c which contains the initiation site described above, whereas capped RNA was strongly hybridizing with this clone. However, as already mentioned by Mulligan *et al.* [22], capped RNA also hybridized with clones d and e which is consistent with a precursor-product relationship between the primary transcript and the mature form.

Characterization of processing intermediates

The fact that mitochondrial phosphorylated RNA was hybridizing with different subclones of this transcription unit strongly suggested the presence of processing intermediates. By using primer extension experiments, major processing sites were identified at the 3′ end of each tRNA: when oligonucleotides 8, 10 and 12 were used as primers,

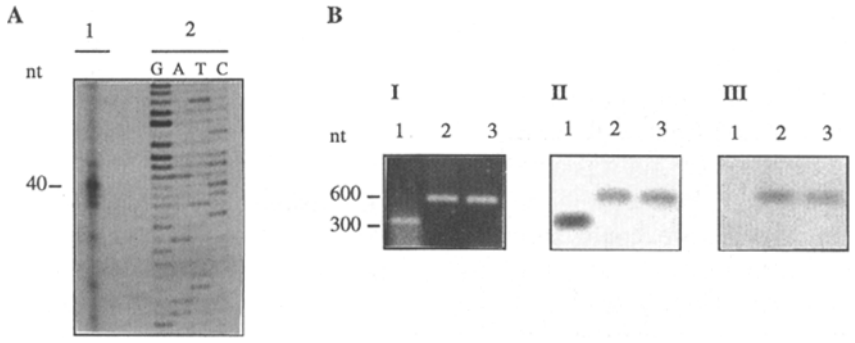

Fig. 4. A. Ribonuclease protection experiment on potato mitochondrial capped RNA using as a probe an antisense transcript synthetized *in vitro* from clone c. The reaction product (1) was loaded on a 6% polyacrylamide gel and a sequence ladder (2) was run in parallel. B. Southern blots hybridization of clones c, d and e digested by *Eco*RI, *Eco*RI/*Hin*dIII and *Hin*dIII/*Acc*I, respectively with (II) potato mitochondrial capped RNA and (III) potato mitochondrial phosphorylated RNA. In (I) is shown the corresponding agarose gel stained with ethidium bromide with the size of the fragments indicated on the left. Name of the clones is according to Fig. 1.

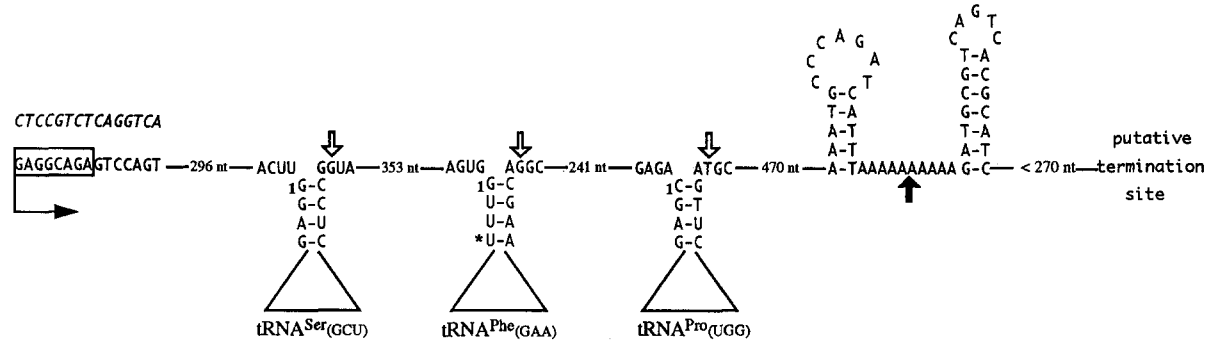

Fig. 5. Schematic representation of the potato mitochondrial primary transcript containing the $tRNA^{Ser}$(GCU), $tRNA^{Phe}$(GAA) and $tRNA^{Pro}$(UGG). The nucleotide sequence corresponding to the transcription initiation region is boxed and indicated by an arrow. The complementary DNA strand is written in italics. The three tRNAs are only schematically represented, their first nucleotide is numbered 1. The ending site found on the $tRNA^{Phe}$(GAA) is noted by an asterisk [19]. Open vertical arrows correspond to stops found by primer extensions at the 3′ end of each tRNA. The black vertical arrow corresponds to another processing site determined by RNase protection. The double loop structure surrounding this site is indicated.

a strong stop was obtained immediately at the 3′ end of $tRNA^{Ser}$, $tRNA^{Phe}$ and $tRNA^{Pro}$ respectively (Fig. 3). The size of the deduced processing intermediates could be correlated with some of the smaller transcripts detected in northern experiments (Fig. 2). For instance, the strong hybridization signals obtained at about 800 nt could be explained by the maturation site found at the 3′ end of $tRNA^{Phe}$ (Fig. 3).

Having localized the transcription initiation site and taking into account the size of the primary transcript, the location of the termination site could be estimated to be roughly 800 nt downstream of *trnP*. This region was subsequently sequenced and is available under the accession number X87676. Furthermore, as the 1800 nt long primary transcript was detected on northern blot when a PCR-DNA probe generated with oligonucleotides 4 and 12 was used, but was undetectable with a PCR-DNA probe corresponding to clone f (data not shown), we could therefore speculate that the termination site is located between the two *Cla*I sites (clone g on Fig. 1). To map this site, ribonuclease protection experiments of the radioactive antisense riboprobe generated from this clone with potato mitochondrial RNA were performed. A 101 nt long protected product was obtained (data not shown) whereas no protected product was detected when total mitochondrial RNA was omitted. As shown on Fig. 5, this region, located 498 downstream of *trnP*, corresponds to a stretch of 10 A residues and is surrounded by a double hairpin structure. Whether this region represents the putative termination site or another maturation site was checked by primer extension experiments. When, either oligonucleotide 13 (32 nt upstream of this site) or oligonucleotide 14 (52 nt downstream of this site) were used as primers, the same extension product, corresponding to the 3′ end of $tRNA^{Pro}$, was found (Fig. 3). According to this result, this structured region corresponds in fact to another processing event that probably occurs not too far from the termination site. The latter site should be located between oligonucleotide 14 and the second *Cla*I site.

Discussion

In this paper, we present a detailed analysis of the *trnS*(GCU), *trnF*(GAA) and *trnP*(UGG) transcription unit of the potato mitochondrial genome. Although tRNA genes are rather dispersed all along the mitochondrial genomes of higher plants [31, 35], we provide here the first evidence for the co-transcription of three potato mitochondrial tRNA genes. The data obtained suggest that

only one initiation site is responsible for this co-transcription. Several primer extension experiments have allowed us to define the transcription start 305 to 312 nt upstream of *trnS* in a purine-rich sequence: GAGGCAGA (Fig. 5). Most of the experiments gave a signal at the A_{305} and/or at the G_{312}. This result demonstrates that transcription of these tRNA genes is directed neither by internal promoters, as in the case of eucaryotic tRNA genes [7] or of a few chloroplastic tRNA genes [10] nor by prokaryotic-like −10 and −35 boxes which are also responsible for the transcription of another class of chloroplastic tRNA genes [9]. Furthermore, except for the fact that this region corresponds to a purine-rich domain, it does not fit with the consensus motif CRTAaGaGA found in mRNA and rRNA promoters of dicotyledonous plants [4] and also recently identified upstream of the *Oenothera* mitochondrial *trnF*(GAA) [3]. These data suggested for the first time that mRNAs, rRNAs and tRNAs could be transcribed by the same RNA polymerase or the same transcription system in plant mitochondria. By contrast, in potato mitochondria, the co-transcription of these three tRNA genes, as well as the transcription of the *atp*9 gene [15] and of the *rrn*26 gene [5], are initiated in unrelated sequences which do not present any homology with the proposed consensus motif for plant mitochondrial promoters. This could mean that different RNA polymerases and/or transcription initiation factors are present in plant mitochondria. As shown by the nine transcription initiation sites found for the *atp*9 gene in maize mitochondria [23] there is, in fact, a considerable variability in the plant mitochondrial promoters and to explain the quite divergent sequence elements identified in potato, we cannot rule out that promoters with different strength are active in plant mitochondria. It also has to be noted that upstream of the *cox*2 gene of teosinte two promoter sequences have been found, one similar to the consensus described for maize mitochondrial promoters and the other one representing a new type of mitochondrial promoter, thus showing that alternate promoters can function in plant mitochondria [24, 27]. In the consensus promoter sequences proposed for monocotyledons or dicotyledons, a CRTA motif was found to be highly conserved ([25] and references therein) and proposed to be necessary for the active transcription of the mitochondrial genes [28]. This CRTA box is absent from the three potato mitochondrial transcription sites mentioned above, however such a motif can be found 22 and 48 nt upstream of the *trnS-trnF-trnP* and *atp*9 promoter regions respectively, but whether it is necessary for the transcription of these genes remains to be determined. Interestingly, we identified a region similar to the promoter site present upstream of the *Oenothera* mitochondrial *trnF*(GAA) within the sequence between the potato mitochondrial *trnS* and *trnF* (Fig. 6). In *Oenothera*, this initiation site fits well with the consensus promoter sequence and is active *in vivo* [3] whereas in the potato sequence there is one nt difference in the CRTA motif and,

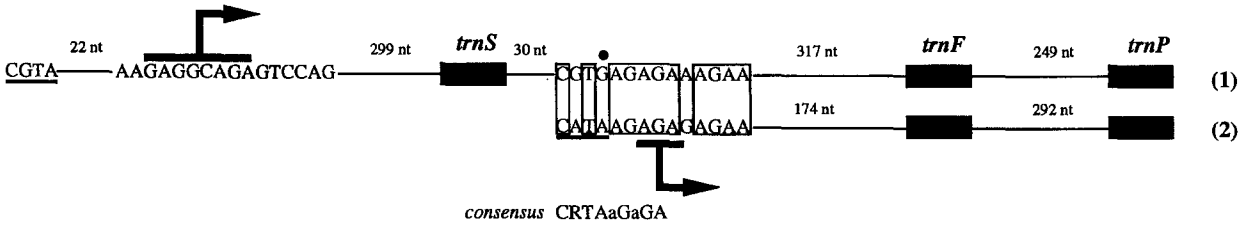

Fig. 6. Schematic comparison between (**1**) the potato mitochondrial transcription unit containing *trnS*(GCU), *trnF*(GAA) and *trnP*(UGG) and (**2**) the corresponding *Oenothera* mitochondrial transcription unit containing *trnF*(GAA) and *trn P*(UGG) [3]. The regions corresponding to the identified initiation sites are shown by arrows. The sequence found 317 nt upstream of the potato mitochondrial *trnF* and showing high homology with the sequence surrounding the *Oenothera* initiation site is written. Sequence homologies between these two regions are boxed. The nucleotide which differs in this potato mitochondrial region from the consensus promoter sequence proposed for dicotyledonous plants [4] is dotted.

as judged by primer extension experiments (Fig. 3), this motif does not seem to act as an initiation site. To really understand the implication of the CRTA motif for tRNA gene transcription with respect to the surrounding sequence, it would be of interest to know whether by changing this nucleotide we can restore initiation of transcription at this site in potato mitochondria.

To excise the three tRNAs from the 1800 nt primary transcript, processing at both 5′ and 3′ ends of each tRNA is required. In order to get more insight on the processing events leading to the mature tRNAs, primer extension experiments were performed using a set of oligonucleotides spanning this transcription unit. Strong and clean stops were obtained at the 3′ end of each tRNA suggesting that, as already shown in the case of the *Oenothera* mitochondrial $tRNA^{Phe}$, the 3′ processing involves a precise endonucleolytic cleavage rather than a 3′ to 5′ exonuclease activity [3]. These results are in agreement with the 3′-endonuclease activity identified by studying the *in vitro* maturation of artificial tRNA precursors in wheat mitochondria [11]. However it was shown using a similar processing system, that the 3′ maturation of at least some *Oenothera* mitochondrial tRNAs could occur via an exonucleolytic activity [16]. Recently, the partial purification of a 3′-tRNA processing activity from potato mitochondria has been reported but it was not defined whether it was an endonuclease or an exonuclease activity [17].

Only few papers are dealing with transcription termination signals in plant mitochondria, but in all of them, secondary structures resembling bacterial terminators have been reported [6, 29, 32]. Using ribonuclease protection experiments, a stretch of 10 A residues surrounded by a double hairpin structure was identified as another processing site located downstream of *trnP*. Taking into consideration the data obtained by northern experiments, one can speculate that the termination site of this transcription unit lies a little bit further this strong secondary structure which could also act as a moderator for the RNA polymerase just before the transcription stops and/or as a stabilizer of the transcript.

The results presented in this paper raise several questions. Is it a general phenomenon that, in potato mitochondria, promoter elements differ from the proposed consensus sequence? Does this imply the existence of different polymerases and/or cofactors? To answer these questions in the case of mitochondrial tRNA genes, it would be useful to study other tRNA gene transcription units and to set up an accurate *in vitro* transcription system not yet available for tRNA genes.

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